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Cultivation of the green macroalgae *Ulva lactuca* and *Ulvaria splendens* for biofuels production

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Abstract

The green macroalgae *Ulva lactuca* and *Ulvaria splendens* are two species particularly interesting in a algal biorefinery concept including biofuel production in Denmark and Greenland, respectively. The possibility of using digested pig manure (DPM) as nutrient source for the cultivation of the two species was investigated, and the macroalgae were also used as substrate for anaerobic thermophilic digestion with DPM as inoculum. Half the algal fronds were washed with fresh water in order to investigate the effect of sea salt in the anaerobic digestion. Furthermore, *Ulva spp*. contains a high amount of carbohydrates (>60%) making them suitable for cellulosic ethanol fermentation. The yeast *Saccharomyces cerevisiae* was used for ethanol fermentation after the enzymatic hydrolysis of the macerated algal biomass, and the effect of alkaline pre-treatment was also investigated in regard to the potential ethanol production. In this study, both species showed potential as candidates for future algal biorefineries using diluted DPM as nutrient source for biomass production and the biomass utilized for biofuels. *Ulva lactuca* had significantly higher growth rates compared to *U.splendens*. However, washed *U.splendens* had significantly higher biogas potential than *U.lactuca*. Results on ethanol concentrations showed that pretreatment of biomass was not necessary prior to fermentation.

The algal biorefinery concept

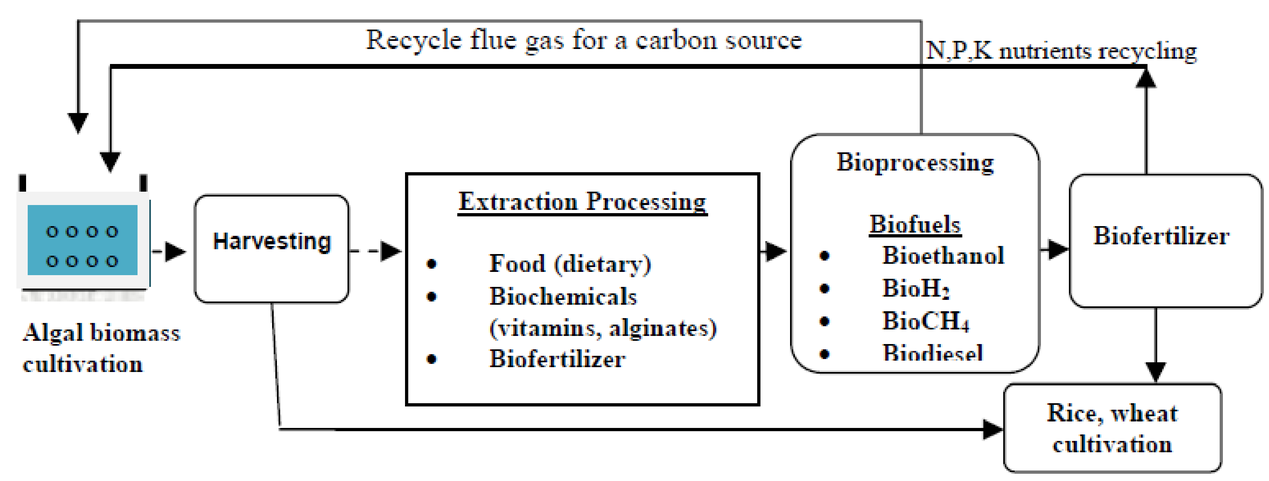


Figure 1: The algal biorefinery concept: Algal biomass cultivated and harvested are utilized for several extracted high value-added products, and biomass waste are converted to biofuels. Waste effluent from biofuel processes, such as digested pig manure are rich in nutrients and may be used for crop or algal cultivation.

Aims

- Review products that can be derived from the green macroalgae *Ulva lactuca* and *Ulvaria splendens.*
- Determine the growth rate of *U.lactuca* and *U.splendens* cultivated under different nutrient regimes.
- Investigate digested pig manure (DPM) as nutrient source candidate for algal growth.
- Quantify the biogas and bioethanol potential production of *U. lactuca* and *U. splendens.*
- Investigate the possible inhibition of the biogas process, by the marine sea salt contained in the superficial seawater present on the algal fronds.
- Determine the alkaline/oxidative pretreatment effect of the macroalgae on the ethanol production.

Materials and methods

Natural populations of *Ulva lactuca* and *Ulvaria splendens* were collected at Danish and Greenlandic shores, respectively, and cultivated in 300 mL aerated seawater enriched with with NO^-_3 (F/2), NH^+_4 (standard algal medium) and diluted (1:200) digested pig manure (mainly NH^+_4) as nitrogen source corresponding to 12.35 mg N/L. Light was provided 24 hours daily with intensity of 48µmolphotons/s/m^2 temperature of 15°C, and experiment duration was 21 d. For experiments of biogas potential 550mL bottles were used with organic loading of 1, 2, and 4 g VS/L macerated algal biomass (washed with fresh water or left with sea water on surface), water and inoculated with digested pig manure to volume of 200mL.. The ethanol potential of the species was measured by HPLC after ezymatic hydrolysis by citric buffer (pH=4.8) and incubation (50°C, 120 rpm, 24h) and fermentation by yeast *Saccharomyces cerevisiae*. Pretreatment consisted of cutting (<2mm) and addition of Mg2SO4 (1g/L), H2O2 (1% V/V) and 0.5 M NaOH for pH adjustment to pH=11.5.

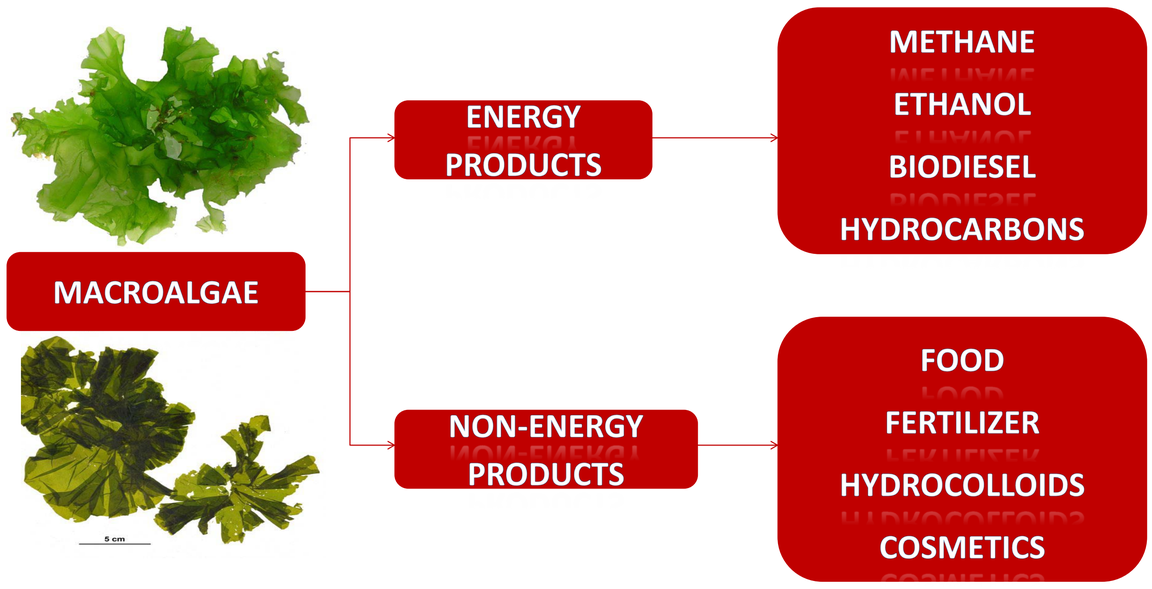
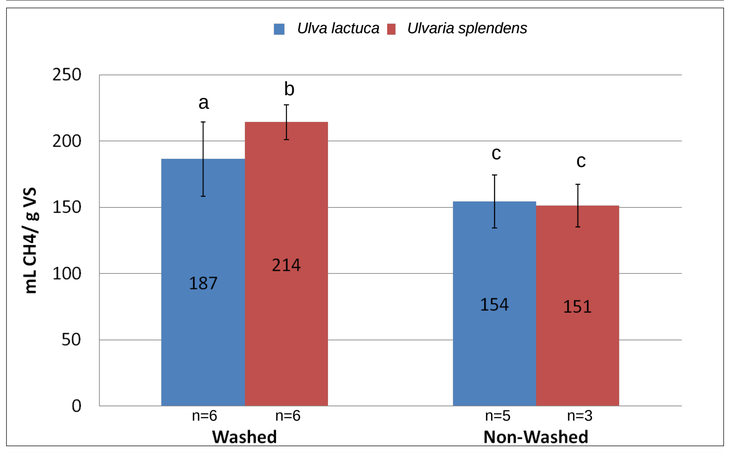
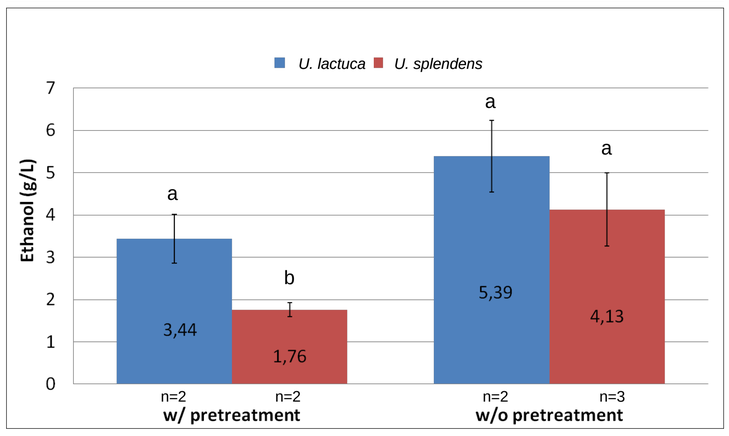


Figure 2: Biofuels and seleced products derived from macroalgae. Photos: *Ulva lactuca* (top), *Ulvaria splendens (*bottom)

Results

Figure 3: Specific growth rates of *U.lactuca* and *U.splendens* cultivated under different nutrient regimes corresponding to 12.35mgN/L. *U. splendens* died after 4days of cultivation in NO^-_3. Different letters represent results of significant difference (n=3, p<0.05)





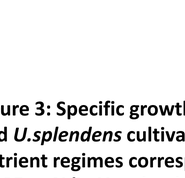
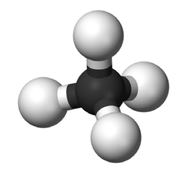
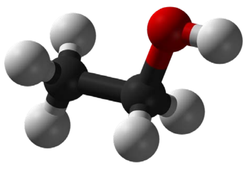


Figure 4: Average methane production of *U.lactuca* and *U.splendens* digested under batch thermophilic conditions. Fresh refers to washed and salt to non-washed samples prior to incubation. Different letters represent results of significant difference (p<0.05)



CH_4

Figure 5: Average ethanol concentrations in the fermentation broth, for alkaline pretreated and non pretreated *U.lactuca* and *U.splendens.* *U.lactuca* had significantly higher concentration of ethanol than *U.splendens* with treatment. The concentration of ethanol was significant higher without compared to with pretreatment for *U.splendens* (p<0.05)



EtOH

- Literature study showed high market potential of carbohydrates (>60% of dry weight including ulvan) derived from especially *Ulva* spp. for various products e.g. cosmetics.
- High growth rates (0.22 d^{-1} with NO_3^- culture medium) and high yields (10 x corn yield).
- *U. lactuca* had higher growth rates for all treatments compared to *U.splendens* (p<0.05).
- Diluted DPM was suitable as nutrient source, however growth rate 27% lower (0.16 d^{-1}) than the maximum achieved.
- Both *U. splendens* and *U.lactuca* could be used for biogas production with a yield of approx. 0.2 L-CH_4/gVS. The CH_4 yields were 21-29% higher when the macroalgae was washed.
- The Na^+ and K^+ concentrations of < 1 g/L should not be inhibiting the biogas process.
- Alkaline/oxidative pretreatment of *U. splendens* decreased the ethanol production significantly, and this species had a significantly lower concentration of ethanol compared to *U.lactuca* when both were pretreated.

Conclusions

Ulva lactuca and *Ulvaria splendens* could both be cultivated on diluted DPM as growth medium, with *U.lactuca* reaching the highest growth rates. The high carbohydrate content of especially *U.lactuca* makes this candidate for ulvan extraction and ethanol fermentation. Pretreatment of biomass was not necessary for biomass fermentation. Higher biogas potentials were reached in the washed *U. splendens* compared to *U. lactuca*. These results make both species candidates for future algal biorefineries.